PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RUTGER 008	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US03/27457	International filing date *(day/month/year)* 04 September 2003 (04.09.2003)	Priority date *(day/month/year)* 04 September 2002 (04.09.2002)
International Patent Classification (IPC) or national classification and IPC IPC(7): G01N 33/53;C12Q 1/37 and US Cl.: 435/7.1, 4, 23, 24		
Applicant RUTGERS, THE STATE UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16 September 2004 (16.09.2004)	14 October 2004 (14.10.2004)
Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Louise N. Leary Telephone No. (571)272-1600

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/27457

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-71 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages 72-79, filed with the demand
pages NONE, filed with the letter of _____.

☒ the drawings:
pages NONE, as originally filed
pages 1-5, filed with the demand
pages NONE, filed with the letter of _____.

☐ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*
*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/27457

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-77	YES
	Claims NONE	NO
Inventive Step (IS)	Claims 1-77	YES
	Claims NONE	NO
Industrial Applicability (IA)	Claims 1-77	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-77 meet all criteria set out under PCT Articles 33(2)-33(4).

---------- NEW CITATIONS ----------

CLAIMS

1. An analog of a peptide comprising an amino acid sequence GGAGHVPEYFVGOGTPISFYG (MccJ25) having an amino acid sequence that differs from MccJ25 in terms of comprising at least one amino acid substitution, insertion, or deletion, and that binds bacterial RNAP and inhibits bacterial RNAP activity to a greater extent than MccJ25.

2. A composition comprising the analog of claim 1 and a carrier.

3. A method for identifying an agent that binds to a homologous bacterial RNAP secondary channel amino acid sequence in a first entity, comprising the steps of: (a) preparing a reaction solution including the agent to be tested and a first entity including the homologous bacterial RNAP secondary channel amino acid sequence; and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the homologous bacterial RNAP secondary channel amino acid sequence.

4. The method of claim 3 wherein the first entity is an intact bacterial RNAP.

5. The method of claim 3 wherein the first entity is a fragment of a bacterial RNAP.

6. The method of claim 3 wherein the bacterial RNAP is derived from *Escherichia* coli.

7. The method of claim 3 wherein the bacterial RNAP is derived from *Bacillus subtilis*.

8. The method of claim 3 wherein said agent is an analog of a peptide comprising an amino acid sequence GGAGHVPEYFVGOGTPISFYG (MccJ25) having an amino acid sequence that differs from MccJ25 in terms of at least one amino acid substitution, insertion, or deletion.

9. The method of claim 3 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to a homologous bacterial RNAP secondary channel amino acid sequence of a second entity that contains a derivative of a homologous bacterial RNAP secondary channel amino acid sequence.

10. The method of claim 9 wherein the second entity is a derivative of an intact bacterial RNAP.

11. The method of claim 9 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

12. The method of claim 9 wherein at least one of the first or the second entities is a derivative of a bacterial RNAP is derived from *Escherichia* coli.

13. The method of claim 9 wherein at least one of the first or the second entities is a derivative of a bacterial RNAP is derived from *Bacillus subtilis*.

14. The method of claim 3 further comprising the step of: detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to a homologous secondary channel amino acid sequence of a second entity that contains a homologous bacterial RNAP secondary channel amino acid sequence of a eukaryotic RNAP or a derivative thereof.

15. The method of claim 14 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

16. The method of claim 14 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

17. The method of claim 9 wherein the second entity is a derivative of a fragment of MccJ25 or MccJ25.

18. The method of claim 14 wherein at least one of the presence, extent, concentration-dependence, or kinetics of

binding of the agent to the entity also is compared to at least one of the presence, extent, concentration-dependence, or kinetics of binding of MccJ25 to the entity.

19. A method for identifying an agent that inhibits an activity of a bacterial RNAP by binding to a homologous bacterial RNAP secondary channel amino acid sequence, comprising (a) preparing a reaction solution comprising the agent to be tested and a first entity containing a homologous secondary channel amino acid sequence; and (b) detecting the at least one of the presence, extent, concentration-dependence, or kinetics of inhibition of an activity of said first entity, wherein inhibition involves binding of the agent to the homologous bacterial RNAP secondary channel amino acid sequence.

20. The method of claim 19 wherein the first entity is an intact bacterial RNAP.

21. The method of claim 19 wherein the first entity is a fragment of a bacterial RNAP.

22. The method of claim 19 wherein the bacterial RNAP is derived from *Escherichia coli*.

23. The method of claim 19 wherein the bacterial RNAP is derived from *Bacillus subtilis*.

24. The method of claim 19 wherein the activity is RNA synthesis.

25. The method of claim 19 wherein the activity is NTP uptake.

26. The method of claim 19 wherein the activity is pyrophosphate release.

27. The method of claim 19 wherein the activity is abortive-RNA release.

28. The method of claim 19 wherein the activity is edited-RNA release.

29. The method of claim 19 wherein the activity is transcriptional pausing.

30. The method of claim 19 wherein the activity is transcriptional arrest.

31. The method of claim 19 wherein the activity is Gre-factor binding.

32. The method of claim 19 wherein determination of the inhibition on binding to the homologous bacterial RNAP secondary channel amino acid sequence includes comparison of: (a) the inhibition by the agent of an activity of the first entity, and (b) the inhibition by the agent of the activity of a second entity that contains a derivative of a homologous bacterial RNAP secondary channel amino acid having at least one substitution, insertion, or deletion.

33. The method of claim 32 wherein the second entity is a derivative of an intact bacterial RNAP.

34. The method of claim 32 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

35. The method of claim 32 wherein the bacterial RNAP is derived from *Escherichia coli*.

36. The method of claim 32 wherein the bacterial RNAP is derived from *Bacillus subtilis*.

37. The method of claim 32 wherein the activity is RNA synthesis.

38. The method of claim 32 wherein the activity is NTP uptake.

39. The method of claim 32 wherein the activity is pyrophosphate release.

40. The method of claim 32 wherein the activity is abortive-RNA release.

41. The method of claim 32 wherein the activity is edited-RNA release.

42. The method of claim 32 wherein the activity is transcriptional pausing.

43. The method of claim 32 wherein the activity is transcriptional arrest.

44. The method of claim 32 wherein the activity is Gre-factor binding.

45. The method of claim 32 wherein inhibition of an activity of the first entity and inhibition of an activity of the second entity are assessed sequentially.

46. The method of claim 32 wherein inhibition of an activity of the first entity and inhibition of an activity of the second entity are assessed simultaneously.

47. The method of claim 19 wherein at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the agent of the activity of the first entity also is compared at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the agent of the activity of a second entity comprising a eukaryotic RNAP derivative.

48. The method of claim 47 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

49. The method of claim 47 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

50. The method of claim 19 or 32 wherein at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the agent of the first entity also is compared to at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by MccJ25 of the first entity.

51. A method for identifying an agent that binds to a homologous bacterial RNAP secondary channel amino acid sequence, comprising (a) preparing a reaction solution

comprising the agent to be tested, a reference compound that binds to a homologous bacterial RNAP secondary channel amino acid sequence, and a first entity containing a homologous secondary channel amino acid sequence, and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of competition by the agent for binding of the reference compound to the homologous secondary channel amino acid sequence.

52. The method of claim 51 wherein the first entity is an intact bacterial RNAP.

53. The method of claim 51 wherein the first entity is a fragment of a bacterial RNAP.

54. The method of claim 52 wherein the bacterial RNAP is derived from *Escherichia* coli.

55. The method of claim 52 wherein the bacterial RNAP is derived from *Bacillus subtilis*.

56. The method of claim 51 wherein the reference compound contains a detectable group.

57. The method of claim 56 wherein the detectable group contains a chromophore.

58. The method of claim 56 wherein the detectable group contains a fluorophore.

59. The method of claim 51 wherein the reference compound is MccJ25.

60. The method of claim 51 wherein the reference compound is a MccJ25 derivative.

61. The method of claim 51 wherein the reference compound is a chromophore-labelled MccJ25 derivative.

62. The method of claim 51 wherein the reference compound is a fluorophore-labelled MccJ25 derivative.

63. The method of claim 51 wherein the reference compound is [Lys_{13}-Cy3]-MccJ25.

64. The method of claim 51 further comprising measurement of FRET.

65. The method of claim 51 wherein determination of the dependence of binding on a homologous bacterial RNAP secondary channel amino acid sequence includes comparison of: (a) the binding by the agent to the first entity, and (b) the binding of the agent to a second entity that contains a derivative of a homologous bacterial RNAP secondary channel amino acid having at least one substitution, insertion, or deletion.

66. The method of claim 65 wherein the second entity is a derivative of an intact bacterial RNAP.

67. The method of claim 65 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

68. The method of claim 65 wherein the bacterial RNAP is derived from *Escherichia* coli.

69. The method of claim 65 wherein the bacterial RNAP is derived from *Bacillus subtilis*.

70. The method of claim 51 or 65 wherein at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity also is compared to at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to a eukaryotic RNAP derivative.

71. The method of claim 70 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

72. The method of claim 70 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

73. The method of claim 70 wherein at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity is compared to at least one of the presence, extent, concentration-dependence, or kinetics of binding of MccJ25 to the first entity.

RPOC_ECOLI	(736)	QIRQLAGMRGLM	(779)	ARK
RPOC_HAEIN	(737)	QIRQLAGMRGLM	(780)	ARK
RPOC_VIBCH	(736)	QIRQLAGMRGLM	(779)	ARK
RPOC_PSEAE	(736)	QIRQLAGMRGLM	(779)	ARK
RPOC_TREPA	(703)	QIRQLAGMRGLM	(746)	ARK
RPOC_BORBU	(699)	QIRQLAGMRGLM	(742)	ARK
RPOC_XYLFA	(759)	QIRQLAAMRGLM	(802)	ARK
RPOC_CAMJE	(734)	QISQLAAMRGLM	(777)	ARK
RPOC_NEIMA	(738)	QIKQLSGMRGLM	(781)	ARK
RPOC_RICPR	(730)	QIKQLGGMRGLM	(773)	MRK
RPOC_THEMA	(1010)	QVKQLAGIRGLM	(1072)	ARK
RPOC_CHLTR	(736)	QLKQLGALRGLM	(779)	ARK
RPOC_MYCPN	(820)	NFTQLFGMRGLM	(874)	ARK
RPOC_BACSU	(740)	NFTQLAGMRGLM	(783)	ARK
RPOC_STAAU	(744)	NFTQLAGMRGLM	(787)	ARK
RPOC_MYCTU	(813)	QTRTLAGMKGLV	(856)	ARK
RPOC_SYNY3	(763)	QVRQLVGMRGLM	(806)	ARK
RPOC_AQUAE	(850)	QIRQLAGMRGLM	(893)	ARK
RPOC_DEIRA	(1052)	QIRQLAGMRGLM	(1095)	ARK
RPOC_TTHER	(1034)	QIRQLCGLRGLM	(1077)	ARK
RPOC_THEAQ	(1034)	QIRQLCGMRGLM	(1077)	ARK
RPA1_HUMAN	(908)	NTMQISCLLGQI	(971)	GRE
RPB1_HUMAN	(780)	NISQVIAVVGQQ	(843)	GRE
RPC1_HUMAN	(791)	NISQMIACVGQQ	(854)	GRE

Bacterial RNA polymerase

Human RNA polymerases I, II, and III

FIG. 1

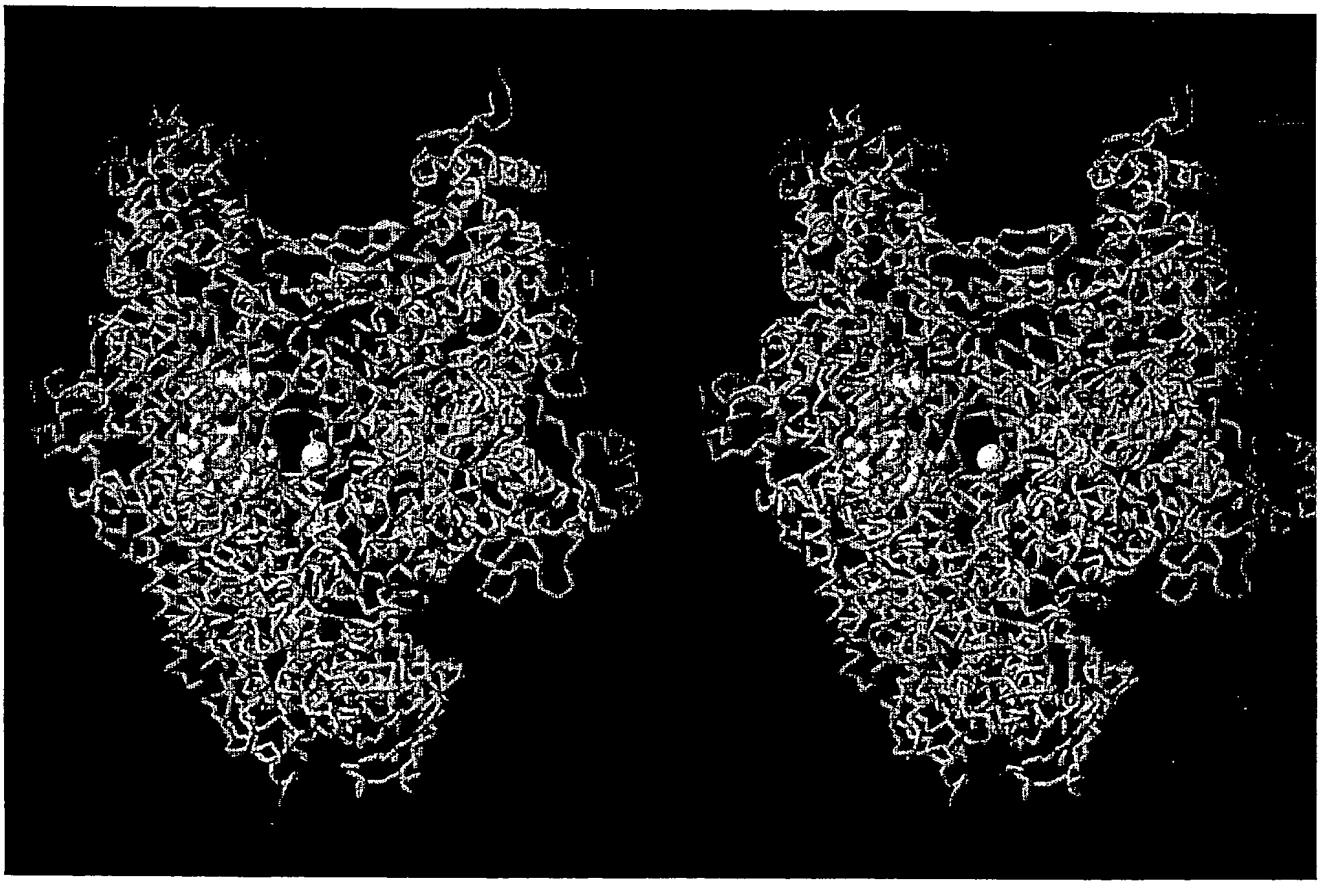

FIG. 2

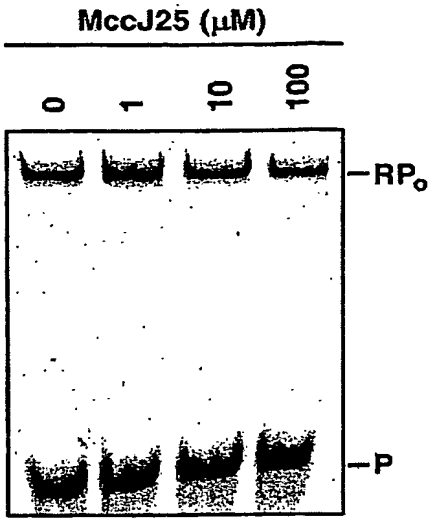

FIG. 3

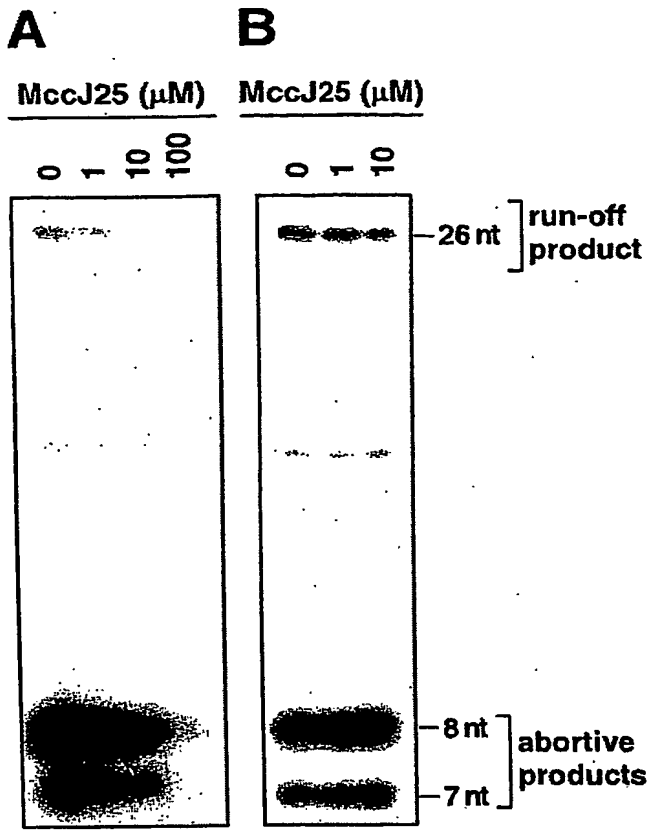

FIG. 4

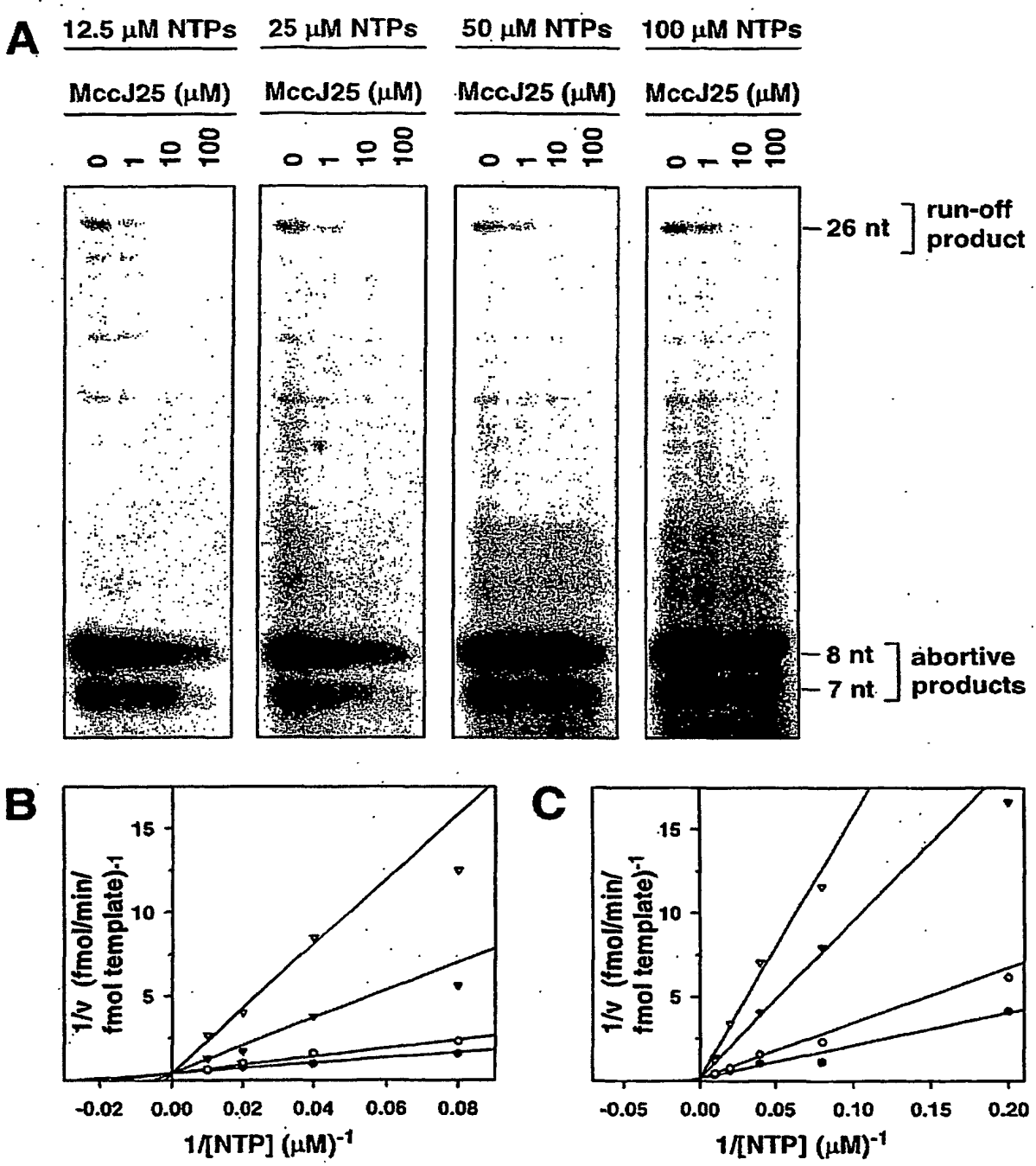

FIG. 5

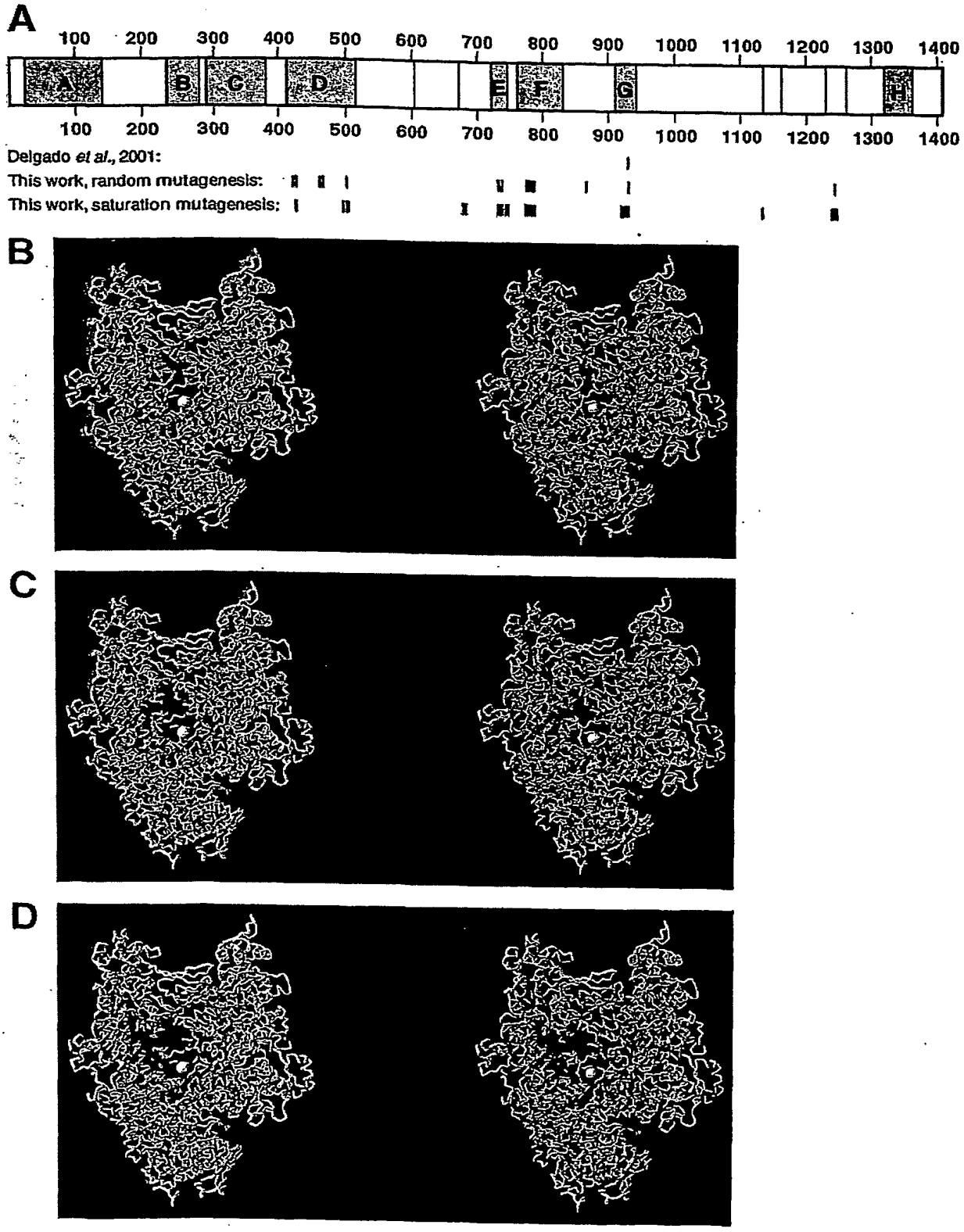

FIG. 6